ACS OMEGA

Article

Cite This: ACS Omega 2018, 3, 18181−18188

http://pubs.acs.org/journal/acsodf

Improving Cell Detachment from Temperature-Responsive Poly(*N*-isopropylacrylamide-*co*-acrylamide)-Grafted Culture Surfaces by Spin Coating

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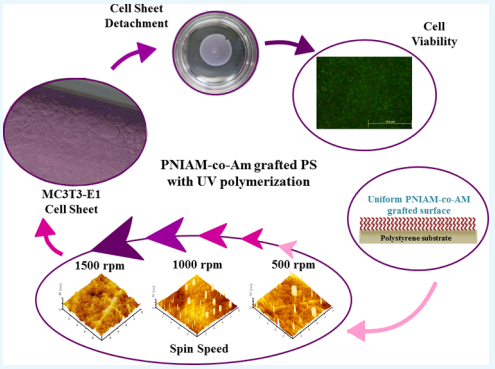
Supporting Information

ABSTRACT: Poly(*N*-isopropylacrylamide-*co*-acrylamide) (PNIAM-*co*-AM)-grafted surfaces have been reported to promote cell adhesion and detachment by a hydrophobic-to-hydrophilic transition triggered by temperature change. However, the surface uniformity and cell detachment consistency are still an issue. In this study, PNIAM-*co*-AM is prepared with spin coating to control the grafting density and the thickness and to achieve better cell detachment. The atomic force microscopy results indicate that the surface becomes smoother as the spin speed increases. The attenuated total reflection Fourier transform infrared results show a grafting density from 1.68 to 2.03 $\mu g/cm^2$. Ellipsometry suggests that the thickness of the spin-coated PNIAM-*co*-AM layer is 11−21 nm. The grafted surfaces were tested with mouse preosteoblast MC3T3-E1 cells, which grew successfully. The detachment reached 100 percent with the samples prepared with 1.5 and 2 h ultraviolet exposure times without the use of a poly(vinylidene difluoride) membrane. The detached sheet was in good condition, as indicated by Live/Dead stains.



1. INTRODUCTION

Cell-sheet engineering is an alternative technology for building a three-dimensional (3D) tissue construct without using 3D scaffolding.[1−6] It is typically done based on temperature-sensitive poly(*N*-isopropyl acrylamide) (PNIAM) to fabricate cell sheets for tissue regeneration.[4−10] The PNIAM surface has a hydrophilic−hydrophobic transition at a lower critical solution temperature (LCST), about 32 °C.[11−13] Below this temperature, the cells can detach from the surface as sheets without using proteolytic enzymes.[14−17] In this case, key structures such as cell−cell junctions and extracellular matrices (ECMs) can be preserved with intact cell monolayers.[18−20]

A temperature-sensitive surface is commercially available under the trademark Upcell.[6] This surface is fabricated by grafting the PNIAM homopolymer onto tissue culture polystyrene (TCPS) surfaces using electron beam (EB) irradiation.[2,21] The mechanism of cell-sheet detachment has passive and active steps.[22,23] Below the LCST, the hydrophilic PNIAM chains extend, resulting in reduced interactions between the cells and grafted culture surfaces in the passive step.[23] This is followed by an active step, in which the cells undergo shape changes due to metabolic processes. However, how the hydrated polymer chains interact with different proteins in the ECM during the detachment is still being investigated. For example, the effects of cell-sheet detachment and ECM adhesion proteins were examined with confluent monolayer bovine aortic endothelial cells (BAECs), whose ECM contains fibronectin, laminin, and collagen type I and type IV.[17,19,24,25] After lowering the temperature, fibronectin and laminin could be recovered, together with the BAECs, whereas the rest of the ECM protein remains on the surface.[24,26] This shows that the type of proteins in ECMs plays a significant role in the detachment process.[23]

Recent studies have focused on attempts to fabricate a temperature-sensitive surface, which can be used for detachment of different cell types.[8,21,27−30] The use of ultraviolet (UV) is a more economical alternative, compared to EB irradiation.[31,32] In addition, it can be used in conjunction with other techniques, such as surface patterning and photolithography techniques, to graft polymers onto TCPS.[33,34]

Received: September 25, 2018
Accepted: December 11, 2018
Published: December 24, 2018

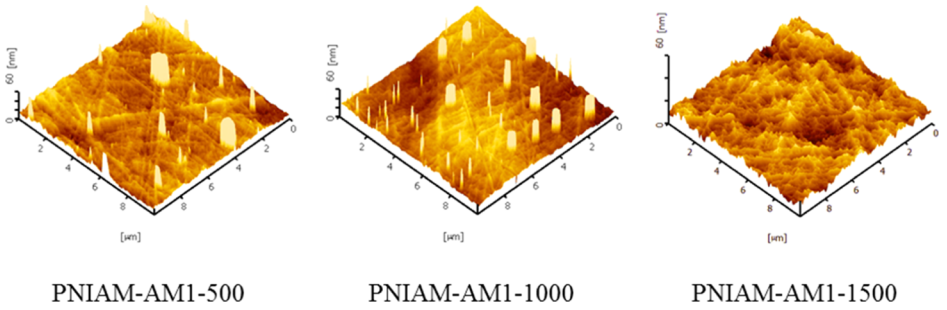


Figure 1. Morphology of spin-coated PNAIAM-*co*-AM at different spin speeds with 1 h UV exposure time.

Our previous studies showed that temperature-sensitive Poly(*N*-isopropylacrylamide-*co*-acrylamide) (PNIAM-*co*-AM) can be grafted onto TCPS by UV irradiation.[32,35] When harvesting cell sheets, the cell monolayers had to be agitated with the culture media. This, however, resulted in 85% detachment of mouse preosteoblast MC3T3-E1 cell sheets. In addition, the detached cell sheets contracted because of strong cell−cell interactions.[35] To prevent cell-sheet shrinkage, a hydrophilically modified poly(vinylidene difluoride) (PVDF) membrane was utilized. It is widely used to support cell lift-off and prevent cell sheets from shrinkage.[36−38] Our study found that the PVDF membrane allowed 96% cell-sheet detachment.[35] However, the cell sheets transferred by the PVDF membrane can be stuck on the membrane because of the protein interaction with the charge on the membrane. Separating a cell sheet from the membrane surface for reattachment can cause the cell sheet to break into smaller pieces. In addition, the use of a PVDF membrane is impractical for clinical use.[39] Although cell sheets could be harvested from the PNIAM-*co*-AM surface, the ability to observe cell−surface interactions cannot be accomplished because of inconsistent cell-sheet detachment and unknown PNIAM-*co*-AM structure properties such as the thickness and the grafting density.

To address the above issues, this study focuses on improving the fabrication of temperature-sensitive surfaces by spin coating, to achieve 100% cell detachment. The use of spin coating allows us to construct consistent PNIAM-*co*-AM-grafted TCPS and to investigate the film thickness and the grafting density.[31,40] We examined two important parameters, namely the spin speed and the UV polymerization time, which affect the grafting density and the polymer thickness.[31,41] These are crucial parameters for controlling cell adhesion and detachment in response to temperature changes.[1,42] Using ellipsometry, we model the polymer structures, which relate to the PNIAM-*co*-AM thickness. We use quantitative analysis in the interpretation of attenuated total reflection Fourier transform infrared (ATR−FTIR) spectroscopy data to evaluate the grafting density, an important factor to allow cell sheets to reach 100% detachment without utilizing a PVDF membrane. Cell viability of the harvested cell sheet is also evaluated for further clinical applications. The ability to examine the PNIAM-*co*-AM surface structure allows us to further study the cell−surface interactions, leading to the ability to engineer different temperature-sensitive surfaces for different cell types.

2. RESULTS AND DISCUSSION

2.1. Characterization of PNIAM-*co*-AM-Grafted TCPS.

Surface topography was investigated by atomic force microscopy (AFM). The surface roughness and the structure images were used to determine the uniformity of the grafted surfaces. The effect of spin speed on the morphologies of the spin-coated surfaces was examined (see Figure 1).

The morphologies of spin-coated surfaces show undulation peaks on the TCPS surface at speeds below 1000 revolutions per minute (rpm). This is unlike PNIAM-AM1-1500 that appears to have better coverage than other samples. The results of the copolymer grafted with a spin speed of 1500 rpm exhibit a more uniform surface, which is consistent with a previous study.[31]

The topography and roughness of the spin-coated surfaces were compared with the ungrafted TCPS and the UpCell plates. In Figure 2a, UpCell shows nanoparticle-like structures with a surface roughness that is slightly lower than that of the ungrafted surface. A decrease of roughness is possibly a result of the polymer uniformly grafted on the surface. In Figure 2b, the phase image of TCPS shows stripes and grooves covering the TCPS surface. The presence of stripes and grooves occurs from the manufacturing process.[43,44] Figure 2c shows the PNIAM-*co*-AM surface prepared by our previous procedure, reported by Wong-In et al.[35] Briefly, PNIAM-*co*-AM-grafted TCPS was fabricated by 1 h UV exposure without spin coating. Figure 2c shows undulation peaks on the TCPS surface. The appearance of stripes and grooves in the phase image suggests that the copolymer prepared without spin coating has nonuniform coverage. In Figure 2d,e, the spin-coated copolymer shows smoother surfaces, similar to the Upcell plate (Figure 2a), where the stripes and grooves of the underlying TCPS are mostly covered. An increase of UV exposure time reduces the surface roughness because of the formation of longer cross-linked copolymer chains. The reported root-mean-square (rms) roughness also suggests that the spin coating and the UV exposure time affect the surface quality, which was found to be smoother than PNIAM-*co*-AM prepared by the previous procedure detailed above.[35] In Figure 2f, higher spin speed (2000 rpm) results in thin copolymer grafted on the TCPS surface. Although the rms roughness decreases as the spin speed increases, the phase image shows stripes and grooves on the surface similar to the ungrafted surface.

Changes in the temperature-dependent wettability of a PNIAM-*co*-AM-grafted surface were investigated, in comparison with the ungrafted TCPS. Figure 3b,c shows that the polystyrene surface is hydrophobic at 10 and 40 °C with the water contact angle (WCA) of 66.3 ± 4.2° and 73.9 ± 1.2°, respectively. However, the wettability of the grafted surface changed as the temperature changed. In Figure 3a, the spin-coated PNIAM-*co*-AM surface is hydrophilic below 32 °C and becomes hydrophobic above this temperature. The contact angle of the water droplet on the grafted surface suddenly decreased at a temperature below 32 °C, which shows the

DOI: 10.1021/acsomega.8b02514
ACS Omega 2018, 3, 18181−18188

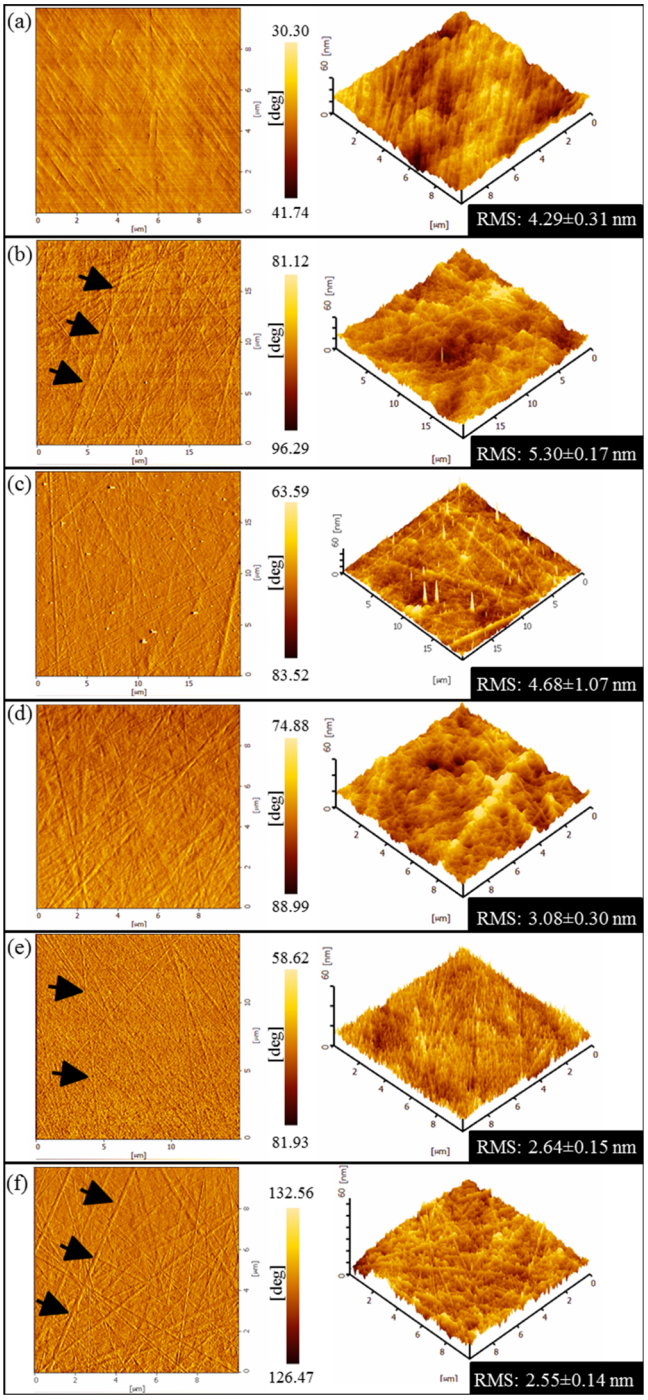


Figure 2. AFM phase image, topography, and rms of (a) ungrafted TCPS. (b) UpCell. (c) PNIAM-*co*-AM prepared by Wong-In et al. (d) PNIAM-AM1-1500. (e) PNIAM-AM2-1500. (f) PNIAM-AM2-2000. The stripes and grooves are displayed as lines on the phase images, specified by the arrows.

temperature responsiveness of PNIAM-*co*-AM (Figure 3d,e). In addition, the water droplet angle at each temperature from the spin-coated samples shows less variation than the PNIAM-*co*-AM prepared by Wong-In et al.[35]

2.2. Effect of Spin Speed and UV Exposure Time on PNIAM-*co*-AM Thickness. PNIAM-*co*-AM-grafted TCPS, following the procedure by Wong-In et al.,[35] cannot be used to evaluate the thickness because of the nonuniform coverage of PNIAM-*co*-AM (Figure 2c). Spin coating provides

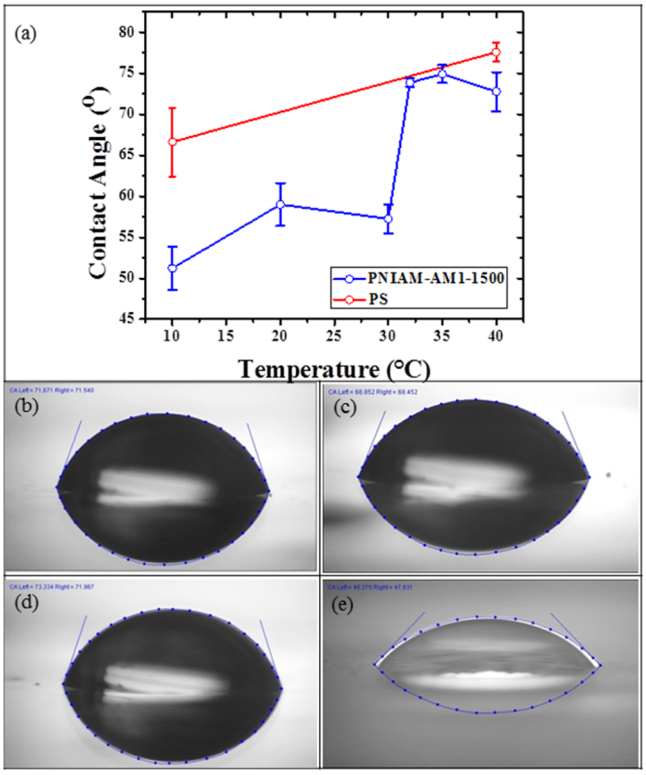


Figure 3. WCA measurement of (a) polystyrene and PNIAM-AM1-1500 at different temperatures. (b,c) Hydrophobic property of TCPS at 40 and 10 °C, respectively. (d,e) Hydrophobic-to-hydrophilic transition of PNIAM-AM1-1500 while temperature is decreased from 40 to 10 °C, respectively.

consistent PNIAM-*co*-AM-grafted TCPS and allows us to generate the thickness model of PNIAM-*co*-AM layers by using ellipsometry. There were a total of two layers: the polystyrene base layer and the PNIAM-*co*-AM layer. The thickness and optical parameters of both layers were modeled by the Cauchy equation. The refractive index of the ungrafted polystyrene was determined to be 1.60 ± 0.01 (λ = 550 nm), which corresponded to the reported value of 1.59−1.60.[45] The changes in the refractive index of PNIAM-*co*-AM were comparatively negligible, approximately 1.56 ± 0.01 under all conditions (Table 1).

Table 1 shows that the increase in the spin speed reduces the copolymer film thickness. The results are consistent with the spin-coating theory.[40] In addition, the grafted copolymer surfaces are more uniform when the spin speed increases, as seen in the decrease of the standard deviation (SD). This agreed with the AFM results; hence, a spin speed of 1500 rpm was selected for the preparation of the surfaces for subsequent experiments.

The effects of UV exposure time on the grafting density and the copolymer thickness were further examined. Spin-coated PNIAM-*co*-AM at 1500 rpm was examined by increasing the UV exposure time. In Table 1, the grafted surfaces with a UV exposure time from 1 to 2 h resulted in a grafting density from 1.68 to 2.03 μg/cm^2, which falls within the valid range of grafting density from 1.4 to 2.0 μg/cm^2, suitable for cell adhesion and detachment.[42] Grafting density increased with increasing UV exposure time, which was consistent with the thickness result. By increasing the exposure time, the number of free radicals formed in the solution increases, which leads to

DOI: 10.1021/acsomega.8b02514
ACS Omega 2018, 3, 18181−18188

Table 1. Grafting Density and Thickness of Spin-Coated PNIAM-*co*-AM at Different Spin Speeds and UV Exposure Times[a]

sample name	UV exposure time (h)	spin speed (rpm)	thickness (nm)	roughness by AFM (nm)	grafting density ($\mu g/cm^2$)	refractive index
PNIAM-AM1-500	1	500	21.77 ± 5.07	15.8 ± 0.8		1.56 ± 0.01
PNIAM-AM1-1000	1	1000	16.52 ± 4.68	12.1 ± 0.5		1.57 ± 0.01
PNIAM-AM1-1500	1	1500	10.77 ± 1.23	3.08 ± 0.30	1.68 ± 0.06	1.56 ± 0.01
PNIAM-AM1.5-1500	1.5	1500	11.97 ± 6.37	4.48 ± 0.79	1.86 ± 0.06	1.57 ± 0.02
PNIAM-AM2-1500	2	1500	16.50 ± 7.76	2.64 ± 0.15	2.03 ± 0.09	1.57 ± 0.01

[a]Thickness is expressed as mean ± SD (n = 3), and grafting density is expressed as mean ± SD (n = 8).

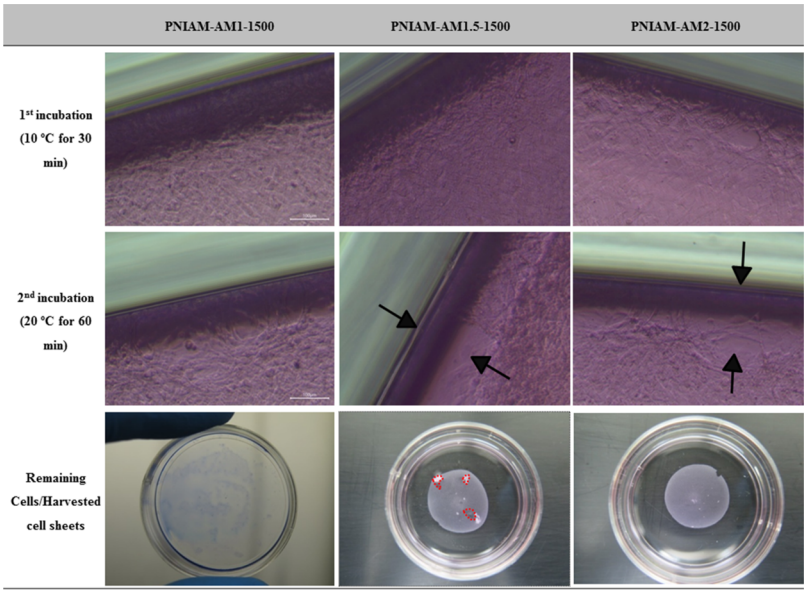


Figure 4. MC3T3-E1 cell-sheet detachment from PNIAM-AM1-1500, PNIAM-AM1.5-1500, and PNAIM-AM2-1500 samples. After the second incubation, the cell sheets spontaneously detached from PNIAM-AM1.5-1500 and PNAIM-AM2-1500. Cells were fully detached from PNAIM-AM2-1500.

Table 2. Percentages of Cell-Sheet Detachment from PNIAM-*co*-AM at 1, 1.5, and 2 h of UV Exposure Time at a Spin Speed of 1500 rpm

condition for cell detachment	percentage of cell detachment		
	PNIAM-AM1-1500	PNIAM-AM1.5-1500	PNIAM-AM2-1500
10 °C (30 min) followed by additional incubation at 20 °C (60 min)	18.5 ± 5.5%	100%	100%

more monomer conversion, resulting in an increase of the grafting yield.[46,47] The thickness of PNIAM-AM2-1500 was 16.50 nm, which falls within the range of 15−30 nm, suitable for cell adhesion and detachment.[1,42]

2.3. Cell Detachment Analysis. Mouse preosteoblast MC3T3-E1 cells were cultured for 3 days to reach 100% confluency, ensuring strong cell−cell junctions. Cell detachment was performed in two steps, as previously established by Wong-In et al.[35] The first step required incubation at 10 °C for 30 min to increase the copolymer chain extension. At this temperature, the cell metabolism was suppressed. As a result, the cells were not able to reorganize their cytoskeletons. This is important for the morphological change and cell detachment.[35] In the second step, the incubation temperature was increased to 20 °C for 60 min to induce the cells to lift-off. Then, MC3T3-E1 cell layers were harvested by gently flushing the surface with the culture medium. The percentages of cell detachment were compared for different UV exposure times.

Figure 4 shows the morphology of the cell sheets, harvested from the surfaces prepared with different UV exposure times, using an inverted microscope. In the first incubation step at 10 °C, all of the cells remained attached to the surface. In the second incubation step, the cells started to detach from the edge of the plate at 1.5 and 2 h of UV exposure. Cell-sheet detachment is seen by the gaps in the walls of the culture well and the cell culture area (black arrows). After detachment, a large number of cells remained well-attached to the surface prepared with 1 h UV exposure, as shown by the color of the trypan blue stain on the temperature-responsive culture well. Harvesting an intact cell sheet was not possible in this condition. However, the complete removal of intact cell sheets was achieved with the PNIAM-*co*-AM film prepared with exposure times of 1.5 and 2 h. The surface prepared with 1.5 h UV exposure yielded a cell sheet with small holes after the detachment, possibly due to the slight agitation from flushing with medium to help the cell sheets to detach completely. In the PNIAM-AM2-1500 sample, MC3T3-E1 cells were completely detached with ease, resulting in tight cell−cell junctions, which are desirable for transplantation. The increase in the grafting density and thickness provided a denser PNIAM-*co*-AM film, which improved the detachment ability of the cell sheets in PNIAM-AM2-1500 samples.

DOI: 10.1021/acsomega.8b02514
ACS Omega 2018, 3, 18181−18188

Table 2 shows the percentages of cell-sheet detachment from spin-coated PNIAM-*co*-AM in different conditions. Without using a PVDF membrane, complete detachment of MC3T3-E1 cell sheets was achieved from the grafted surfaces prepared with 1.5 and 2 h of UV exposure. At 1 h exposure, only 18.5% of the cell sheets were detached. Although the thickness of 1 h exposure was similar to that of 1.5 h UV exposure, the grafting density from 1 h was less than that for 1.5 h of UV exposure. The smaller the amount of copolymer on the grafted surface, the smaller the amount of hydrophilic moieties from the primary and secondary amide groups that were grafted on the surface. This resulted in a low percentage of cell-sheet detachment in the samples with 1 h UV exposure. Nonetheless, complete detachment of the cell sheets was still possible from 1 h UV exposure if a PVDF membrane was utilized.[35]

2.4. Viability of Harvested Cell Sheets. To analyze the growth potential of MC3T3-E1 cells after cell-sheet detachment, the viability of the harvested cell sheets must be considered. The harvested cell sheets were transferred to fetal bovine serum (FBS)-coated TCPS and allowed to attach at 37 °C for 24 h before staining the cell sheets with the Live/Dead stain. Figure 5 shows Live/Dead stained images of the cell sheets from PNIAM-AM1.5-1500 and PNIAM-AM2-1500.

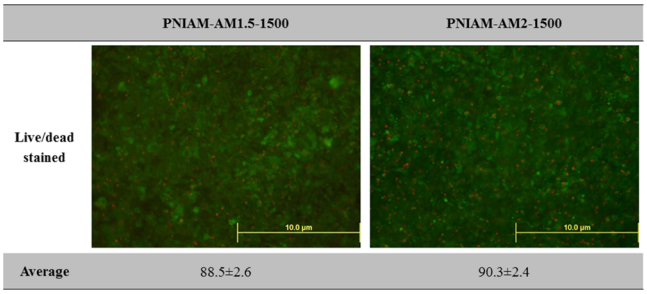


Figure 5. Percentage of viable MC3T3-E1 mouse preosteoblast cells after detachment from PNIAM-AM1.5-1500 and PNAIM-AM2-1500 surfaces. Viable cells are shown in green, whereas dead cells are shown as red dots.

The cell viabilities of PNIAM-AM1.5-1500 and PNIAM-AM2-1500 were 88.5 ± 2.6 and 90.3 ± 2.4%, respectively. Only a few dead cells in red dots are observed in Figure 5, indicating that the cell sheets detached from both conditions were healthy and in good quality. PNIAM-AM2-1500 was suggested for the construction of MC3T3-E1 cell sheets, as it yielded complete removal of intact cell sheets with high cell viability, as described previously.

3. CONCLUSIONS

In this study, the preparation method of grafting PNIAM-*co*-AM onto TCPS surfaces was improved by using the spin-coating technique. Successful polymerization was confirmed by AFM and WCA measurements. AFM images show the topography of more uniform surfaces compared to the previously established method. Ellipsometry showed that the optimal thickness of the grafted surface can be achieved at a spin speed of 1500 rpm. The effective UV polymerization time was found to be from 1 to 2 h, which provided a suitable grafting density of 1.68−2.03 $\mu g/cm^2$. MC3T3-E1 cell detachment supported the thickness and grafting density results because 100% of MC3T3-E1 cells were detached from a spin-coated PNIAM-*co*-AM surface prepared with 1.5 and 2 h UV exposure times with a spin speed of 1500 rpm. Complete removal of the cell sheets was achieved with a denser PNIAM-*co*-AM film prepared with 2 h UV exposure. Furthermore, the viability of MC3T3-E1 cell sheets detached from the samples prepared with 1.5 and 2 h UV exposure times were more than 88 and 90%, respectively.

4. MATERIALS AND METHODS

4.1. Materials. *N*-Isopropylacrylamide (NIAM) was purchased from Aldrich (Sigma-Aldrich, St. Louis, MO) and was recrystallized at −5 °C in *n*-hexane. Acrylamide (AM) was purchased from Merck (Kenilworth, NJ). *N*,*N*′-Methylenebisacrylamide (MBAM) and potassium periodate (KIO_4) were purchased from Aldrich (Sigma-Aldrich, St. Louis, MO). TCPS dishes, each 35 × 10 mm^2 (Falcon 3001, BD Bioscience, Billerica, MA), were used without any further treatment.

4.2. Preparation of PNIAM-*co*-AM-Grafted Surface. A TCPS dish was submitted to an oxidation process by using a UV lamp (UVGL-58, 6 W, and 254 nm) for 30 min.[48] After oxidation, an aqueous solution of 30 μL containing NIAM (1 mol L^{-1}), AM (1.04 mol L^{-1}), cross-linker MBAM (20 mmol L^{-1}), and photoinitiator KIO_4 (5 mol L^{-1}) were added to each TCPS dish. A spin coater, constructed by using a cooling fan, connected to a power supply (CORSAIR CO-9050007-WW) was used to spread the solution uniformly on the surface using a spin speed of 500, 1000, and 1500 rpm for 5 min. The dishes were immediately exposed to UV with three different exposure times: 1, 1.5, and 2 h. The PNIAM-*co*-AM-grafted TCPS dishes were kept at room temperature under vacuum for 24 h, then washed with ethanol three times to remove the unreacted monomers, and dried in a vacuum oven for another 24 h. PNIAM-*co*-AM-grafted surfaces are abbreviated as PNIAM-AM*X*-*Y* where *X* is the UV irradiation time in hours, and *Y* is the spin speed in rpm, as shown in Table 1. The samples were further characterized to obtain a relationship between the thickness of the copolymer film and the amount of the grafted copolymer, as a function of the spin speed and the UV polymerization time.

4.3. Characterization of PNIAM-*co*-AM-Grafted TCPS. An atomic force Seiko Instrument SPA 400 microscope (tapping mode in air) was used to measure the surface topography. A cantilever SI-DF3 with a spring constant of 1.7 N m^{-1} and a resonant frequency of 10 kHz was used. The surface images were taken at a scan rate of 0.5 Hz. The samples PNIAM-AM1-500, PNIAM-AM1-1000, and PNIAM-AM1-1500 were examined to observe the topography of their surfaces. The ungrafted TCPS and UpCell, commercial temperature-sensitive surfaces, were used as the control.

PNIAM-*co*-AM-grafted surfaces were also analyzed using WCA measurement to observe the change of their surface wettability as a function of temperature. The temperature dependence of PNIAM-*co*-AM surfaces was examined by using an OCA 40 Video-Based Contact Angle Meter. A grafted surface was kept on a temperature-controlled device with temperatures ranging from 10 to 40 °C. The samples were allowed to equilibrate for 15 min before each measurement. The image of a water droplet on each sample was captured at three different positions, and the averages of the reported values of the contact angles was computed.

4.4. Thickness Measurement. The thickness of the PNIAM-*co*-AM layer was characterized by a rotating-analyzer spectroscopic ellipsometer (J.A. Woollam Co. VASE2000).

DOI: 10.1021/acsomega.8b02514
ACS Omega 2018, 3, 18181−18188

The measurements were performed at an incidence angle of 55° in the wavelength range of 300−1200 nm at 10 nm intervals. The values of ρ_π and ρ_σ were measured and described in the ellipsometric ratio, ρ, as eq 1

$$\rho = \frac{\rho_\pi}{\rho_\sigma} = \tan(\psi)e^{i\Delta} \quad (1)$$

where ρ_π and ρ_σ are the complex reflection coefficients of the parallel and perpendicular polarized light components, respectively. The optical model, based on the Cauchy function $\left(n = An + \frac{Bn}{\lambda^2} + \frac{Cn}{\lambda^4}\right)$, was used to extract the information of PNIAM-*co*-AM thickness and refractive index with the regression analysis method. In addition, the samples required a preparation step to remove the effect of reflection at the backside of the substrate.

4.5. Grafting Density Measurement. ATR in combination with FTIR was used to identify the characteristic peaks of the PNIAM-*co*-AM-grafted surfaces and to quantify the amount of grafted copolymer on TCPS. The ATR accessory was equipped with a diamond ATR crystal. The surfaces were placed over the ATR crystal and measured by single-bounce ATR−FTIR (iD7 Nicolet iS5, Thermo Scientific) spectroscopy. Quantitative analysis of PNIAM-*co*-AM compared the area ratio of the secondary amide groups from PNIAM-*co*-AM (wavenumber of 1654 cm^{-1}) with the benzene groups from polystyrene (wavenumber of 1600 cm^{-1}). The area ratio of these two peaks ($y = A_{1654}/A_{1600}$) was determined at eight different positions. Grafting density was determined by using a standard curve.

4.6. Cell Detachment Analysis. A mouse preosteoblast MC3T3-E1 cell line (passage 10−20 for all experiments) was provided by the Faculty of Medicine, Chulalongkorn University (Thailand). MC3T3-E1 cells (1×10^6 cells/mL) were seeded onto sterilized PNIAM-*co*-AM-grafted 35 mm culture dishes in Dulbecco's modified Eagle medium supplemented with 10% FBS and 1% penicillin and streptomycin (Invitrogen, USA). The dishes were incubated for 3 days at 37 °C under a CO_2 (5%) atmosphere to promote cell attachment and spreading. For cell detachment, nonadherent cells were removed by washing with 2 mL of phosphate-buffered saline. Fresh medium (4 mL) was added to the culture plates, followed by low-temperature incubation at 10 °C for 30 min and 20 °C for 60 min.[35] The cell layers were harvested by gently agitating the surface with the culture medium. The cell morphology in each well plate was observed by an inverted microscope (Sundrew MCXI600, Vienna, Austria). The undetached cells were stained with trypan blue to identify the areas of cell detachment.

4.7. Viability of the Harvested Cell Sheets. The detached cell sheets were transferred to FBS-coated TCPS surfaces. The cell sheets were allowed to attach to the new surfaces at 37 °C for 24 h. Once attached, the cell sheets were stained with the Live/Dead Assay Kit (L-3224, Invitrogen) and observed under a fluorescence microscope (Olympus BX60) to determine the cell viability. Viable cells are shown in green, whereas dead cells are observed as red dots. Cell viability was expressed as percent survival, as shown in eq 2

$$\%\ \text{Cell viability} = \frac{\text{live cells (green)}}{\text{total cells number(live cells + dead cells)}} \times 100 \quad (2)$$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02514.

Additional WCA measurement and parameters for the optical model based on the Cauchy equation (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge the contributions of the members of the OTL lab (Optical Thin Film Technology Lab) at the National Electronics and Computer Technology Center (NECTEC). This work is supported by the Construction and Maintenance Technology Research Center—CONTEC at SIIT, which provided a Center of Excellence (CoE) Scholarship, and the Thammasat University Research Fund grant (grant no. TP 2/23/2560).

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